

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 1 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**Contents**

A.	INTRODUCTION .....	2
B.	EQUIPMENT .....	5
C.	REAGENTS AND SOLUTIONS .....	7
D.	STANDARDS .....	8
E.	EXTRACTION PROCEDURE .....	9
F.	ANALYTICAL PROCEDURE.....	10
G.	CALCULATIONS.....	13
H.	HAZARD ANALYSIS .....	13
I.	QUALITY ASSURANCE PLAN .....	14
J.	WORKSHEET .....	18
K.	REFERENCES .....	20

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 2 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**A. INTRODUCTION**

1. Theory

Moxidectin, ivermectin and doramectin are potent anthelmintics used in food animals to control parasitic infections. Moxidectin, ivermectin and doramectin extracted from tissue with acetonitrile; extraneous substances are removed using alumina chromatographic cleanup. All analytes are determined by HPLC after formation of a fluorescent derivative product with trifluoroacetic anhydride/1-methylimidazole. Action levels vary with species/tissue. The method is rapid, has high extraction efficiency and produces derivatized extracts suitable for direct analysis by HPLC.

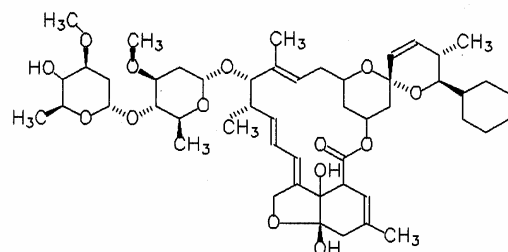
2. Applicability

Tissues/species of interest are liver and muscle in bovine, ovine, porcine, caprine and equine species. This method meets sensitivity requirements and has been validated for all species/tissue combinations.

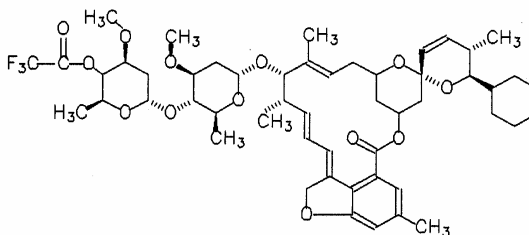
**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>	Page 3 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>	
Revision: .01	Replaces: IVR/DOR1, October, 1998
	Effective: 7-26-02

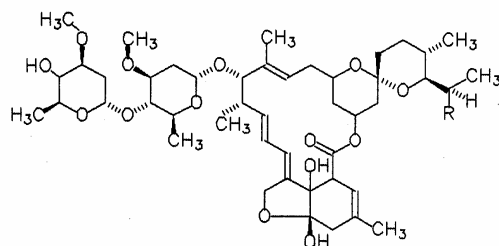
2. Structures



Doramectin

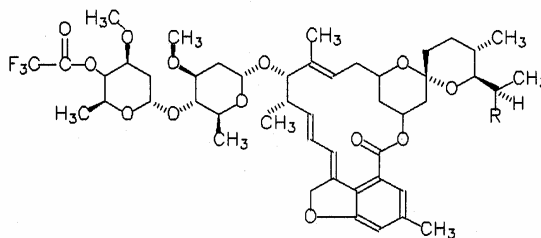


Doramectin Derivative



Ivermectin

R = C<sub>2</sub>H<sub>5</sub> for H<sub>2</sub>B<sub>1A</sub>  
R = CH<sub>3</sub> for H<sub>2</sub>B<sub>1B</sub>



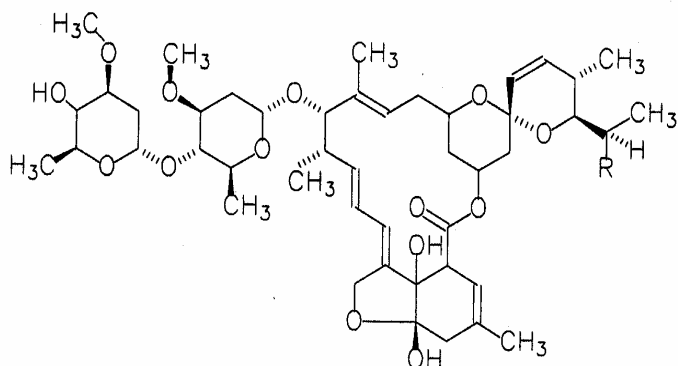
Ivermectin Derivative

R = C<sub>2</sub>H<sub>5</sub> for H<sub>2</sub>B<sub>1A</sub>  
R = CH<sub>3</sub> for H<sub>2</sub>B<sub>1B</sub>

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

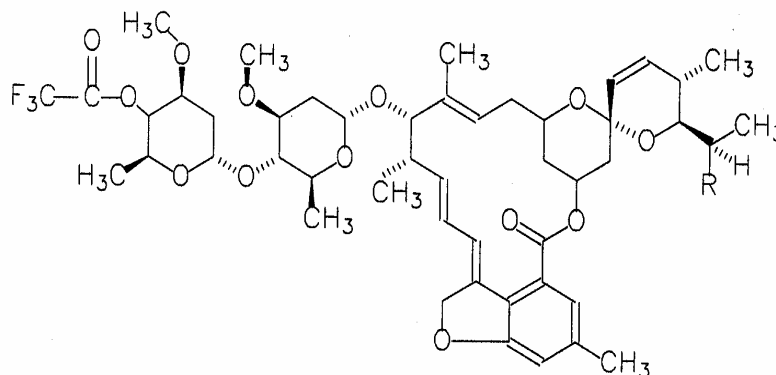
SOP No: CLG-AVR.01		Page 4 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

2. Structures (cont.)



**Abamectin**

R = C<sub>2</sub>H<sub>5</sub> for H<sub>2</sub>B<sub>1A</sub>  
R = CH<sub>3</sub> for H<sub>2</sub>B<sub>1B</sub>



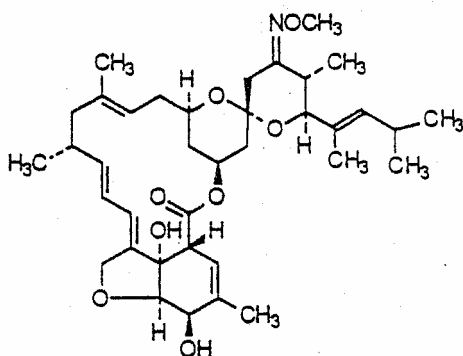
**Abamectin Derivative**

R = C<sub>2</sub>H<sub>5</sub> for H<sub>2</sub>B<sub>1A</sub>  
R = CH<sub>3</sub> for H<sub>2</sub>B<sub>1B</sub>

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 5 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

2. Structures (cont.)



Moxidectin

**B. EQUIPMENT**

1. Apparatus

- a. N-EVAP – model 112, Organomation Assoc. Inc., Berlin MA 01503.
- b. Centrifuge – Sorvall model T-6000B, DuPont Co., Newton, CT 06470.
- c. Mechanical shaker – Eberbach model 610 equipped with shaker box model 6040. Thomas Scientific, Swedesboro, NJ, 08065-0099.
- d. Vortex mixer – Fisher Scientific, Fisher Scientific, Norcross, GA, 30091.
- e. Extraction columns - Fisher Scientific Prep Sep-R (empty) Cat. No. P449R, Fisher Scientific, Norcross, GA, 30091.
- f. 50 mL screw cap centrifuge tubes – Fisher Scientific #05-558-12B, Fisher

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 6 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

Scientific, Norcross, GA, 30091.

- g. 50 mL polypropylene centrifuge tubes – Evergreen Scientific 222-3937-G80, Evergreen Scientific International Inc., Los Angeles CA, 90058-0248.
- h. EDP Plus Micropipet, Rainin Instruments Inc., Emeryville, CA.
- i. Eppendorf Pipettor 4789, Brinkman Instrument Inc., Westbury, New York.
- j. Eppendorf Combitips – 5.0 mL and 12.5 mL, Brinkman Instrument Inc., Westbury, New York.
- k. SPE Cartridges – Place a small silanized glass wool plug into the neck of a 5.75" disposable transfer pipet. Add 0.1 ±0.01 g C18 bulk packing material into the disposable pipet. Tap gently to settle.

NOTE: An equivalent apparatus may be substituted for any of the apparatus above.

2. Instrumentation

a. Liquid Chromatographic System

- i. Waters Model 510 HPLC Pump, Waters Associates, Milford, MA.
- ii. Waters model 717 WISP injection system, Waters Associates, Milford, MA 01757.
- iii. Waters column temperature control module and heater. Waters Associates, Milford, MA 01757.
- iv. Waters 474 Fluorescence detector, Waters Associates, Milford, MA 01757.
- v. Waters model 746 Data Module, Waters Associates, Milford, MA 01757.
- vi. Zorbax ODS 4.6 mm x 15 cm C18 analytical column, DuPont Co., Wilmington, DE 19898.
- vii. Brownlee Labs Spheri-5 RP-18, 30 mm x 4.6 mm 5 micron guard column, Applied Biosystems, Inc. Foster City, CA 94404.
- viii. Alltech Solvent Recycler 2000.

NOTE: An equivalent instrument may be substituted for any of the above.

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 7 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**C. REAGENTS AND SOLUTIONS**

1. Reagents and Solutions

- a. Acetonitrile LC Grade
- b. Alumina-Neutral type WN-3, Activity grade 1, Sigma Chemical Co., St. Louis, MO 63178. Dry at  $135 \pm 5$  °C for at least 24 hours prior to use.  
  
Prepare deactivated alumina for column chromatography. Alumina should be 12% deactivated. For example: Add 24 g distilled/deionized water to 176 g alumina. Mix by shaking until there are no visible lumps. Store deactivated alumina at room temperature in a tightly closed container. Use within one week after opening.  
  
Prepare alumina columns by weighing  $2.0 \pm 0.2$  grams of deactivated alumina into an empty Prep-Sep column.
- c. 1-methylimidazole - redistilled (99+ %), Aldrich Chemical Co. Cat. No. 33,609-2, Aldrich Chemical Co., Inc., Milwaukee, WI 53233.
- d. Trifluoroacetic anhydride (TFA) - (99+ %) Aldrich Chemical Co. Cat. No.10,623-2, Aldrich Chemical Co., Inc., Milwaukee, WI 53233.
- e. Methanol - LC Grade.
- f. Derivatizing reagents
  - i) 1 -methylimidazole - 1: 1 v/v 1-methylimidazole/acetonitrile.
  - ii) TFAA - 1: 1 v/v trifluoroacetic anhydride/acetonitrile.
- g. Liquid chromatograph mobile phase: 97% methanol / 3% mL water v/v.
- h. Sylon CT, Supelco # 3-3065, Supelco, Inc., Bellefonte, PA, 16823-0048.
- i. Waters Bondapak C18 bulk packing material, 125 Å, 37 - 55 µm, Cat. No. WATO30632.
- j. Glass Wool Silane treated.

NOTE: An equivalent reagent or solution may be substituted for any of the above.

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 8 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**D. STANDARDS**

1. Source

- a. Ivermectin standard catalog no. L-640,471-076P004  
Merck, Sharpe and Dhome  
Rahway, NJ 07065
- b. Abamectin standard catalog no. L-676-863-038A003  
Merck Sharpe and Dhome  
Rahway, NJ 07065
- c. Doramectin standard  
Pfizer  
Lee-Summit, MO 64081-2998
- d. Moxidectin standard-catalog no. 301423  
American Cyanamid Company  
Princeton, NJ 08543

2. Preparation of Standards

- a. Follow manufacturer's instructions accompanying standards to obtain a stock solution of approximately 125  $\pm$ 1  $\mu$ g/ml in acetonitrile.
- b. Make a 1:250 dilution to obtain a spiking solution of 0.5  $\mu$ g/ml in acetonitrile.

3. Storage Conditions

- a. Store stock solution in freezer.
- b. Spiking solutions may be stored at room temperature.

4. Shelf Life Stability

- a. Spiking solution: 90 days
- b. Stock: 1 year



**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 9 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**E. EXTRACTION PROCEDURE**

- a. Weigh  $2.5 \pm 0.2$  gram ground tissue into a 50-ml polypropylene centrifuge tube.
- b. Add  $8 \pm 0.5$  ml acetonitrile and vortex for  $30 \pm 5$  seconds.
- c. Fortify each sample with  $150 \pm 2\mu\text{L}$  of  $0.5 \mu\text{g/mL}$  (equivalent to 30 ppb) abamectin internal standard solution. Fortify moxidectin, ivermectin and doramectin recoveries with appropriate volume of  $0.5 \mu\text{g/mL}$  spiking solution depending on species analyzed.
- d. Centrifuge for 3 min  $\pm$  30 sec. at  $1500 \pm 100$  RPM.
- e. Pour acetonitrile eluent through deactivated alumina column and collect eluate in a 50-ml glass centrifuge tube.
- f. Repeat extraction with additional  $8 \pm 0.5$  ml acetonitrile, centrifuge and decant through alumina column combining eluents.
- g. Evaporate acetonitrile under a gentle stream of dry nitrogen or dry air at approx.  $65 \pm 5$  °C.
- h. Reconstitute the dried sample from step (g) using  $0.5 \text{ ml} \pm 50\mu\text{l}$  acetonitrile. Vortex to mix.
- i.
  - (1) Muscle tissue—Add  $2 \pm 0.2$  ml acetonitrile and proceed to m.
  - (2) Liver tissue—Prepare SPE C18 cartridge by placing a small silanized glass wool plug into the neck of a 5.75" disposable pipet. Add  $0.1 \pm 0.01$  g of C18 bulk packing material into the disposable pipet on top of the glass wool. Tap gently to settle C18. This SPE clean-up step for liver tissue eliminates co-extractants that interfere with quantitation.
- j. Pre-wet the SPE cartridge with  $1.0 \pm 0.1$  ml acetonitrile. Discard the wash.
- k. Load the 0.5-ml sample, from step h, onto the wet SPE cartridge. **(The columns must not dry out at any time or the Ivermectin recoveries will be low).** Collect the eluent.
- l. Add  $2 \pm 0.2$  mL of acetonitrile to the sample tube. Mix. Add to the SPE column. Collect the eluent in the same container as the initial 0.5 ml eluent.  
  
Note: Properly dispose of the SPE column.
- m. Add 200  $\mu\text{l}$  1-methylimidazole/acetonitrile reagent a to the eluent and vortex at least 10 sec.
- n. Add 200  $\mu\text{l}$  TFA/acetonitrile reagent and vortex at least 10 sec.
- o. Inject on HPLC.

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 10 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

NOTE: Derivatized samples decompose on exposure to strong light. Allow the sample to derivatize in the dark for approximately 15 minutes before HPLC analysis.

**F. ANALYTICAL PROCEDURE**

1. Instrumental conditions

Note: System may be adjusted to insure optimum response.

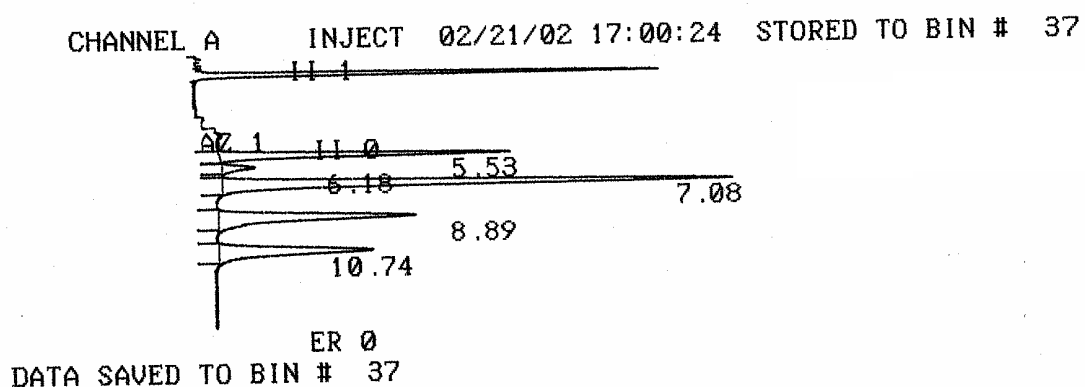
- a. Flow rate: 1.8 ml/min.
- b. Column temp. 30°C.
- c. Injection volume: 50 µL – As determined by detector/integrator conditions.
- d. Run time: 15 min.
- e. Detector settings
  - i. Excitation wavelength 365 ± 20 nm
  - i. Emission wavelength 465 ± 20 nm
- f. Detector gain: As system conditions dictate to ensure approximately ½ scale peak heights.

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: CLG-AVR.01		Page 11 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

2. Sample Chromatograms

a. 15 ppb Standard (Ivermectin, Abamectin, Doramectin, and Moxidectin).



IVERMECTIN SCREEN 02/21/02 17:00:24 CH= "A" PS= 1.  
 FILE 1. METHOD 2. RUN \*\*\* INDEX 1 CALIB BIN 37  
 ANALYST: DMH

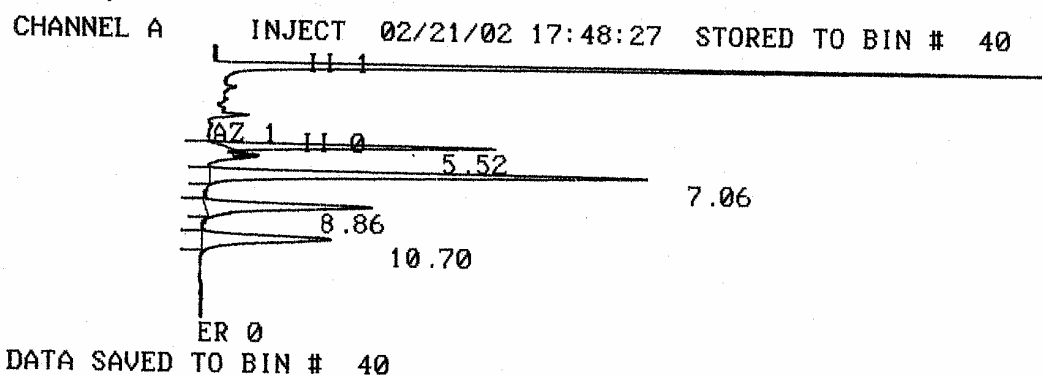
NAME	PPB	RT	PK HT BC	RF	RRT
MOXIDECTIN	15.	5.53	459 01		0.781
ABAMECTIN INTERNAL STD		7.08	809 01		1.
DORAMECTIN	15.	8.89	309 01		1.256
IVERMECTIN	15.	10.74	241 01		1.517
TOTALS	46.		1818		

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: CLG-AVR.01		Page 12 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

2. Sample Chromatograms (con't)

b. 15 ppb Beef Liver Recovery (Ivermectin, Abamectin, Doramectin, and Moxidectin).



IVERMECTIN SCREEN 02/21/02 17:48:27 CH= "A" PS= 1.

FILE 1. METHOD 2. RUN \*\*\* INDEX 1 BIN 40

ANALYST: DMH

SAMPLE 1 REC BL BIN 40 NAME ARUN1734

SA IS XF  
1. 1. 1.

NAME	PPB	RT	PK HT BC	RF	RRT
MOXIDECTIN	15.814	5.52	421 01	26.643	0.782
ABAMECTIN INTERNAL STD		7.06	701 01	1.	1.
DORAMECTIN	14.807	8.86	265 01	17.874	1.255
IVERMECTIN	14.662	10.7	203 01	13.868	1.516
TOTALS	45.283		1591		

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 13 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**G. CALCULATIONS**

Quantitation is performed by measuring peak heights. Each set is accompanied by an external standard curve at 15, 30, and 60 ppb.

Measure peak height of abamectin, ivermectin, moxidectin, and doramectin peaks in the standards and calculate the peak height ratios.

$$\text{Peak Height Ratio} = \frac{\text{Moxidectin, Ivermectin or Doramectin Peak Height}}{\text{Abamectin Peak Height}}$$

Construct a linear regression line using the ratios and standard concentrations. The correlation coefficient should be >0.995.

The equation is  $y = mx + b$

x = Ivermectin, Moxidectin, or Doramectin /Abamectin peak height ratio

y = Ivermectin, Moxidectin, or Doramectin concentration (ppb)

m = slope

b = y-intercept

Incurred tissue ivermectin, moxidectin, or doramectin concentrations should be calculated using this regression line.

**H. HAZARD ANALYSIS**

1. Method Title — DETERMINATION OF IVERMECTIN, DORAMECTIN, and MOXIDECTIN RESIDUES IN ANIMAL TISSUES.
2. Required Protective Equipment — Safety glasses, appropriate gloves, lab coat.
3. Hazards

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 14 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

<i><b>Reagents</b></i>	<i><b>Hazard</b></i>	<i><b>Recommended Safe Procedures</b></i>
Acetonitrile, Trifluoroacetic anhydride, 1-methylimidazole	Flammable and corrosive, may cause skin or respiratory irritation.	Avoid contact or prolonged exposure to vapors. Work in a fume hood. Keep away from flame or heat.
Ivermectin Abamectin	Weak teratogen and possible mutagen	Handle with extreme caution.
Doramectin	Severe explosion hazard if in powdered form.	Handle with extreme caution.
Moxidectin	May cause skin or respiratory irritation. The toxic effects of this material have not been fully evaluated.	Work in a well ventillated area. Store material in a secure, dry, cool well ventillated room.

4. Disposal Procedures

Dispose of solvents according to local, state, and federal guidelines.

**I. QUALITY ASSURANCE PLAN**

1. Performance Standard

<i>Analyte</i>	<i>Analytical Range</i>	<i>Acceptable Recovery</i>	<i>Acceptable Repeatability (CV)</i>
Ivermectin	≥ 7.5 ppb	60-120	< 20
Doramectin	≥ 7.5 ppb	60-120	< 20
Moxidectin	≥ 7.5 ppb	60-120	< 20

Regression coefficient ≥ 0.995

The Measurement Uncertainty and Method Detection Limit should be recalculated yearly

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 15 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

or whenever a change that affects method accuracy, precision, or sensitivity occurs.

2. Critical Control Points and Specifications

***Record***

***Acceptable Control***

- |                               |                     |
|-------------------------------|---------------------|
| a. Sample weight              | 2.5 g. $\pm$ 0.2 g. |
| b. Alumina deactivation level | 12%                 |
| c. 1-methylimidazole volume   | 200 $\mu$ l         |
| d. TFA volume                 | 200 $\mu$ l         |

3. Readiness To Perform

a. Familiarization

i. Phase I:

Standards- Standard curves on each of 3 consecutive days, which will include the following:

- (a) 0 ppb
- (b) 7.5 ppb
- (c) 15 ppb
- (d) 30 ppb
- (e) 60 ppb

ii. Phase II:

Fortified samples- 3 replicates at 0,  $\frac{1}{2}$  x, x, and 2x where x represents the action level for each species (bovine, ovine, and porcine) over a period of 3 different days.

NOTE: Phase I and Phase II may be performed concurrently.

iii. Phase III:

Check samples for analyst accreditation.

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 16 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

- (a) 15 samples fortified between 0 and 60 ppb.
    - (b) Samples submitted by the Quality Assurance Manager (QAM) or supervisor
    - (c) Letter from QAM is required to commence official analysis.
- 4. Acceptability criteria.
  - i. Correlation coefficient > 0.995 for all three days.
  - ii. Mean recovery for each species in range of 60-120%.
  - iii. Repeatability for each species < 20%.
  - iv. No false positive or false negative results.
- 5. Intralaboratory Check Samples
  - a. System, minimum contents.
    - i. Frequency: One check sample per week per analyst as samples analyzed.
    - ii. Blind check samples: Samples chosen at random by supervisor or QAM .
    - iii. Records are to be maintained by the analyst and reviewed by the supervisor and QAM for:
      - (a) All replicate findings.
      - (b) CUSUM and/or SHEWHART charts.
      - (c) All recovery values.
      - (d) Running average, standard deviation, and CV for all recoveries.
  - b. Acceptability criteria.

If unacceptable values are obtained, then:

    - i. Stop all official analyses by that analyst.
    - ii. Take corrective action.
- 6. Sample Acceptability and Stability
  - a. Matrices: Liver, Muscle



**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 17 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

- b. Species: Bovine, porcine, ovine, caprine and equine.
  - c. Sample size: 16-oz minimum.
  - d. Condition on receipt in Sample Preparation Section: frozen.
  - e. Sample Storage:
    - i. Time: 6 months
    - ii. Condition: Frozen
- 7. Sample Set
  - a. Standards at 15, 30 and 60 ppb.
  - b. Recovery at x level.
- 8. Sensitivity
  - a. Lowest detectable level (LDL): 2 ppb.
    - i. Lowest reliable quantitation (LRQ): 7.5 ppb.
    - ii. Minimum proficiency level (MPL): 7.5 ppb.

**United States Department of Agriculture  
Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 18 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**J. WORKSHEET**

The worksheet on the next page can be removed from this book for photocopying.

SOP No: <b>CLG-AVR.01</b>		Page 19 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

Regression Output: Liver Curve				
	MOX	DOR	IVR	
Y Intercept				
Slope				
R				
No. of Observations	4	4	4	4
Degrees of Freedom	2	2	2	2

Regression Output: Muscle Curve				
	MOX	DOR	IVR	
Y Intercept				
Slope				
R				
No. of Observations	4	4	4	4
Degrees of Freedom	2	2	2	2

<b>Analysis Parameters</b>	
500ng/ml Combined Std:	Iv, Dora, Moxi
Date Prepared:	
500 ng/ml Internal Std:	Abamectin
Date Prepared:	

<b>With 500 ng / mL conc.:</b>
Spiking volume of 75 $\mu$ L = 15 ppb
Spiking volume of 150 $\mu$ L = 30 ppb

Critical Control Points	
Sample weight:	2.5 +/- 0.2g
1-Methylimidazole volume:	200 ul +/- 10 ul
TFAA Volume:	200 ul +/- 10 ul
Final dilution volume:	2.5 ml

LC Parameters	
LC Column(ODS):	15cm X 4.6mm
Mobil Phase:	97:3, MeOH:H <sub>2</sub> O
Flow Rate:	1.8 ml/min
Injection Volume =	
Injection Volume =	

Blank Used	
Liver ID Number	
Mouse ID Number	

	PPB	MOX	ABA	DOR	IVR	M/A	D/A	I/A
	Blank	0				0	0	0
	L Std1	15						
	L Std2	30						
	L Std3	60						
	Blank	0				0	0	0
	M Std	15						
	M Std	30						
	M Std	60						

Sample Information	
Analyte Code:	990
Analyst Name:	
Analyst ID:	
Analyst Code:	
Date Started:	
Date Comp'd:	
Check PPB:	
Rec PPB:	

Avermectin codes
Moxidectin: 992
Doramectin: 991
Ivermectin: 923

[illegible]

## Avermectin Screen Worksheet

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 20 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

**K. References**

DeMontigny, Pierre, Jung-sook, K. Shiem and Pivnichny, J. V., J. Pharm. and Biochem. Anal., Vol. 8 No. 6, pp. 507-511, (1990).

Doherty, Steven J., Fox, Allen and Fink, David W., J.A.O.A.C. Vol. 73, No. 6, pp. 931-934, (1990).

Prabhu, Sunil V., Wherner, Teresa A., Egan, Richard S. and Tway, Patricia C., J. Agric. Food Chem., Vol. 39, pp. 2226-2230, (1991).

**United States Department of Agriculture**  
**Food Safety and Inspection Service, Office of Public Health Science**

SOP No: <b>CLG-AVR.01</b>		Page 21 of 21
Title: <b>DETERMINATION OF IVERMECTIN, DORAMECTIN, AND MOXIDECTIN RESIDUES IN ANIMAL TISSUES</b>		
Revision: .01	Replaces: IVR/DOR1, October, 1998	Effective: 7-26-02

Approved by:

Date:

Eric Flynn

7-22-02

Gina McLeroy

7-17-02

Bill Koscinski

7-22-02

Jess Rajan

7-17-02